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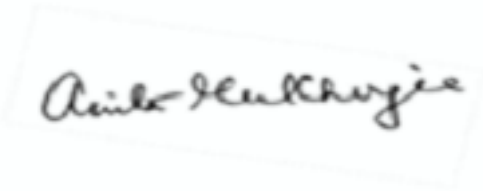
Selection of high yielding elite chemotypes of paclitaxel producing Himalayan yew (*Taxus wallichiana*) from different geographical regions of the Himalayas



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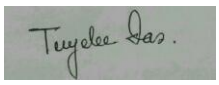
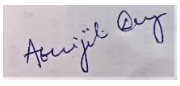
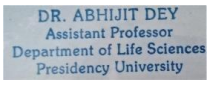




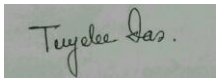
Selection of high yielding elite chemotypes of paclitaxel producing Himalayan yew (*Taxus wallichiana*) from different geographical regions of the Himalayas

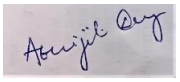
Abstract

The significance of medicinal plants has no limits in traditional and pharmacological industries. Himalayan yew or *Taxus wallichiana* Zucc. synonym *Taxus baccata* subsp. *wallichiana* (Zucc.) Pilg. is profoundly investigated and examined throughout the world due to the presence of one of the most miraculous anti-cancer drug paclitaxel and different other extremely important secondary metabolites. Paclitaxel trade name Taxol® is effective against breast, lung, head, neck, bladder, ovary, and cervix cancers. All species of *Taxus* including *T. wallichiana* grow slowly throughout the year. Therefore, extraction and isolation of paclitaxel for industrial uses from Himalayan yew are the most challenging as paclitaxel is present in very low amount in bark, stem, and leaves. Therefore, its increasing demands in cancer treatment and restricted supply of paclitaxel have eventually expanded the expense of the medication. Hence, this study focuses on the investigation of the quantitative analysis of marker compound (paclitaxel) from Himalayan yew from different locations that can determine the most potent Himalayan yew chemotypes collected from different altitude of Himalayas. Additionally, this present work focuses on the identification of some other phytochemicals and bioactivities of different of various plants parts. Therefore, aims to standardized and validated HPTLC (high performance thin layer chromatography) protocol for quantitative analysis of phytomarker anticancer compound. In addition, it also presents some phytochemical analysis and pharmacological properties of different plant extract investigation on the proposed area will contribute to scientific utilization.

Introduction

Natural plant-derived chemicals have become popular for the treatment of several diseases. This increases the overwhelming interest of researchers in indigenous plants that can complement conventional health care system. Ethnomedicinal studies have shown various scopes in developing a number of drugs to solve multiple challenges in the health sector. Even though every plant species is a valuable, useful, and versatile source of phytochemicals, crediting to their healing property, plant families belonging to gymnosperm are known for their unique and important phytochemicals





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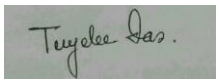
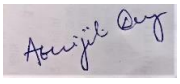
with curative properties against different diseases. Taxaceae family belongs to gymnosperm which contains a large number of terpenoids mostly diterpenoids with anticancer activity. Our plant of interest is *T. wallichiana* or Himalayan yew.

Cancer causes death of more than 9.6 million individuals worldwide in 2018. The marker compound of this selected medicinal plant is paclitaxel, a type of taxane, which has been reported extensively for its wide application in cancer research. Paclitaxel is an alkaloidal diterpenoid that has been found to be efficacious against several human cancers. As yew is a slow-growing plant and the presence of taxane in it very low (0.001-0.05%), it hampers commercial production of this anti-cancer drug (Kumar et al., 2019). Additionally, phytochemical content varies according to the season, time, geographical location, and altitude. The present study thus focuses primarily on the selection of high-yielding Himalayan yew chemotypes in terms of paclitaxel content. Beside this present study also aims to find out other phytochemicals and toxicological aspects of different chemotypes.

Brief literature review: One of the oldest living things on the globe is yew. Yew is a perennial gymnosperm traditionally used for against diarrhea, cold, epilepsy, headaches, hysteria, cough, fever, falling pulse, feebleness, giddiness, and nervousness etc. (Aslam and Reshi, 2017; Idolo et al., 2010; Juyal et al., 2014; Phondani et al., 2010; Purohit et al., 2001; Sharma and Uniyal, 2010; Wahab et al., 2016). It is a very rich source of other diterpenoids, alkaloids, anthraquinones, essential oils, lignans, saponins, steroids, phenols, esters, and flavonoids (Gauchan, 2018; Jenniskens et al., 1996; Labossiere and Thompson, 2018; Ma et al., 1994; Verdian- Rizi et al., 2010; Zarek and Waligórski, 2009). *Taxus* spp. are also known for their bioactive ingredients present on bark that useful as an anti-cancer, anti-microbial, anti-inflammatory, and antinociceptive activities (Bernaitis et al., 2013; Küpeli et al., 2003; Nisar et al., 2008; Juyal et al., 2014). A correlation study revealed negative relation between paclitaxel content and rainfall (Salmore and Hunter 2001). Despite all these important properties of yew, the plant has also been reported for its toxicity. Only red arils of fruit are eaten raw (Wilson et al., 2001). High throughput phytochemical and pharmacological assessment on this plant might pave way for the discovery of other bioactive compounds. Keeping the above information in view, the objectives of the present study are as follows:





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Objectives:

1. Collection and identification of *T. wallichiana* from the Himalayas.

2. Identification of elite chemotypes in terms of paclitaxel production from different Himalayan population of *T. wallichiana* using a validated HPTLC method.

3. Quantification of altitudinal, seasonal, and tissue specific variation of paclitaxel based on different chemotypes.

4. Qualitative and/or quantitative detection of some other phytochemicals from the selected chemotypes.

5. Genotoxicity assessment of the various plant extracts and pure compound.

Plan of work with methodology

Objective 1:

Work plan 1a: Collection of leaves, stem, and bark from Himalayan yew from different populations and altitudes of the Himalayas.

1b: Identification of the *T. wallichiana* with correct taxonomical methods and with proper authentication by taxonomist. Preparation and deposition of herbarium for future reference.

Objective 2:

Work plan 2a: Extraction and quantification of plant materials using different methods, parameters and solvent system.

Objective 3:

3a: Quantification and validation of the marker compound paclitaxel for altitudinal, seasonal, and tissue specific variations using HPTLC. Chromatographic validity will be assayed in terms of specificity, linearity, accuracy etc.

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Objective 4:

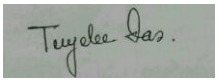
Work plan 4a: Extraction of different plant parts using various solvents for some other phytochemicals such as alkaloid, flavonoid, phenolic, ash content, etc.

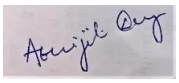
Objective 5:

Work plan 5a: Genotoxic assessment of the plant extract using *Allium cepa* root tip assay via assessment of possible chromosomal aberrations (*viz.* anaphase bridges, chromosomal fragmentation, laggard chromosomes, multi-polarity, etc.).

Significance of the study:

Over-exploitation, low germination, and slow-growing properties of *T. wallichiana* has limited phytochemicals extraction from its parts for industrial uses. However, in the present research work, standardization of HPTLC protocol for the marker compound and method validation based on specificity, linearity, accuracy etc. will not only contribute to understand the thorough experimental knowledge of different chemotypes for the first time but will also provide detailed idea about the anticancer compound paclitaxel content in several *T. wallichiana* populations that further will help the pharmaceutical industries to select elite *T. wallichiana* chemotypes for further use. Toxicological assessment (*Allium cepa* test) of *T. wallichiana* chemotypes will help to find out occurrence of possible genotoxicity of *T. wallichiana* in plant system.





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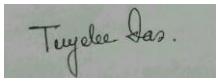

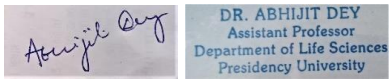

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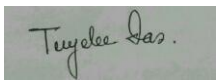

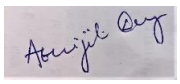

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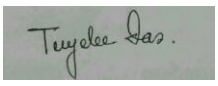

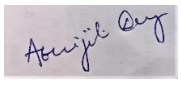

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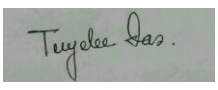
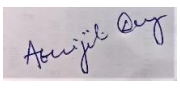
Abstract The significance of medicinal plants has no limits in traditional and pharmacological industries. Himalayan yew or Taxus wallichiana Zucc. synonym Taxus baccata subsp. wallichiana (Zucc.) Pilg. is profoundly investigated and examined throughout the world due to the presence of one of the most miraculous anti-cancer drug paclitaxel and different other extremely important secondary metabolites. Paclitaxel trade name Taxol® is effective against breast, lung, head, neck, bladder, ovary, and cervix cancers. All species of Taxus including T. wallichiana grow slowly throughout the year. Therefore, extraction and isolation of paclitaxel for industrial uses from Himalayan yew are the most challenging as paclitaxel is present in very low amount in bark, stem, and leaves. Therefore, its increasing demands in cancer treatment and restricted supply of paclitaxel have eventually expanded the expense of the medication. Hence, this study focuses on the investigation of the quantitative analysis of marker compound (paclitaxel) from Himalayan yew from different locations that can determine the most potent Himalayan yew chemotypes collected from different altitude of Himalayas. Additionally, this present work focuses on the identification of some other phytochemicals and bioactivities of different of various plants parts. Therefore, aims to standardized and validated HPTLC (high performance thin layer chromatography) protocol for quantitative analysis of phytomarker anticancer compound. In addition, it also presents some phytochemical analysis and pharmacological properties of different plant extract. investigation on the proposed area will contribute to scientific utilization. Introduction Natural plant-derived chemicals have become popular for the treatment of several diseases. This increases the overwhelming interest of researchers in indigenous plants that can complement conventional health care system. Ethnomedicinal studies have shown various scopes in developing a number of drugs to solve multiple challenges in the health sector. Even though every plant species is a valuable, useful, and versatile source of phytochemicals, crediting to their healing property, plant families belonging to gymnosperm are known for their unique and important phytochemicals for curing different diseases. Taxaceae family belongs to gymnosperm which contains a large number of terpenoids mostly diterpenoids with anticancer activity. Our plant of interest is Taxus baccata subsp. wallichiana (Zucc.) Pilg. or Himalayan yew. This plant is a slow-growing and long-living tree, reaching maturity only at 70 years. Cancer causes death of more than 9.6 million individuals worldwide in 2018. The marker compound of this selected medicinal plant is paclitaxel, a type of taxane, which has been reported extensively for its wide application in cancer research. Paclitaxel is an alkaloidal diterpenoid that has been found to be efficacious against several human cancers. As yew is a slow-growing plant and the presence of taxane in it very low (0.001-0.05%), it hampers commercial production of this anti-cancer drug (Kumar et al., 2019). Additionally, phytochemical content varies according to the season, time, geographical location, and altitude. The present study thus focuses primarily on the selection of high-yielding Himalayan yew chemotypes in terms of paclitaxel content. Beside the present study also aims to find out other phytochemicals and biological activities of different chemotypes. Brief literature review: One of the oldest living things on the globe is yew. Yew is a perennial gymnosperm traditionally used for against diarrhea, cold, epilepsy, headaches, hysteria, cough, fever, falling pulse, feebleness, giddiness, and nervousness etc. by ethnic groups such as Rajis, Bhoxas, Bhotias, Gujars, Jads, Jaunsaris, Dard, Kashimiries, and Pathans (Aslam and Reshi, 2017; Idolo et al., 2010; Juyal et al., 2014; Phondani et al., 2010; Purohit et al., 2001; Sharma and Uniyal, 2010; Wahab et al., 2016). Except for paclitaxel, it is a very rich source of other diterpenoids, alkaloids, anthraquinones, essential oils, lignans, saponins, steroids, phenols, esters, and flavonoids (Gauchan, 2018; Jenniskens et al., 1996; Labossiere and Thompson, 2018; Ma et al., 1994; Verdian-Rizi et al., 2010; Zarek and Waligórski, 2009). Taxus spp. are also known for their anti-cancer, anti-microbial, anti-inflammatory and, antinociceptive properties (Bernaitis L et al., 2013; Küpeli et al., 2003; Nisar et al., 2008). The bark of the yew plant is broadly collected for bioactive ingredients (Juyal et al., 2014). A correlation study revealed negative relation between paclitaxel content and rainfall (Salmore and Hunter 2001). Despite all these important properties of yew, the plant has also been reported for its toxicity. Only red arils of fruit are eaten raw (Wilson et al., 2001). High throughput phytochemical and pharmacological assessment on this plant might pave way for the discovery of other bioactive compounds. Keeping the above information in view, the objectives of the present study are as follows:

Objectives: 1. Collection and identification of T. wallichiana from The Himalayas.2. Quantification of paclitaxel as marker compound from different chemotype of T. wallichiana using a validated HPTLC method. 3. Investigation and identification some other phytochemicals from the selected chemotypes. 4. Assessment of bio-activity of plant extract derived from different plant parts (leaf, stem, bark etc). 5. Genotoxicity assessment of the various plant extract. Plan of work with methodology Objective 1: Collection of leaves, stem, and bark from Himalayan





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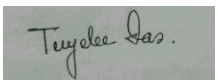
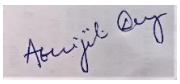
yew from different populations and altitude of Himalayas. Objective 2a: Extraction of plant materials using different solvents standardization, parameters and solvent system. Objective 2b: Quantitative HPTLC study and method validation for the marker compound paclitaxel. Chromatographic validity will be assayed in terms of specificity, linearity, accuracy etc. Objective 3: Extraction of different plant parts using various solvent for identification some other phytochemicals of flavonoid, phenolic, ash content, etc. Objective 4: Assessment of bioactivity or pharmacological activity of various plant extract in terms of anti-microbial. anti-oxidant and other assays. Objective 5: Genotoxic assessment of the plant extract using Allium cepa root tip assay.





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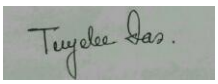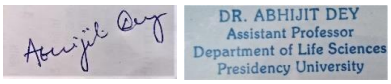
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